

This listing of claims will replace all prior versions, and listings, of claims in the application:

**LISTING OF CLAIMS**

1. *(currently amended)* A method of determining the relative copy number (CN) of a first nucleotide sequence I (NucSeqI) in a sample using an amplification technique, said method comprising the steps of:
  - (1) adding to the sample nucleotides, primers, polymerase, ~~a fluorescently-labeled probe~~probe directed to NucSeqI and NucSeqI, comprising a first fluorophore and a quencher, and optionally, any additional reagents required for amplification, wherein the sample comprises a chromosome-derived second nucleotide sequence II (NucSeqII) and a probe directed to NucSeqII and NucSeqII' comprising a second fluorophore and a quencher, wherein the first fluorophore and the second fluorophore are different;
  - (2) performing one or more amplification cycles to amplify the NucSeqI, carrying out the following amplification steps:
    - (a) amplifying NucSeqI,
    - (b) amplifying NucSeqII,
    - (c) amplifying a third nucleotide sequence I' (NucSeqI') corresponding to NucSeqI and present in a control sample at multiple dilutions, wherein the relationship of NucSeqI and NucSeqI' is defined as
      - (A) NucSeqI hybridizes to the complement of NucSeqI', and
      - (B) NucSeqI' hybridizes to the complement of NucSeqI,both under stringent hybridization conditions, and if NucSeqI and NucSeqI' differ in length, the shorter of the two is at most 30% shorter than the other; and
    - (d) amplifying a fourth nucleotide sequence II' (NucSeqII'), corresponding to NucSeqII and present in a control sample, at multiple dilutions, wherein the relationship of NucSeqII and NucSeqII' is defined as
      - (A) NucSeqII hybridizes to the complement of NucSeqII', and
      - (B) NucSeqII' hybridizes to the complement of NucSeqII,

both under stringent hybridization conditions, and if NucSeqII and NucSeqII' differ in length, the shorter of the two is at most 30% shorter than the other;

wherein

- (i) the ratio of concentration of NucSeqI' to the concentration of NucSeqII' is known,
  - (ii) standard curves SC<sub>I</sub> and SC<sub>II</sub> comprising at least two reference points are generated by amplification of NucSeqI' and NucSeqII', respectively, at multiple dilutions,
  - (iii) at least one pair of amplification reactions (a) and (b) or (c) and (d) is performed in a single container and monitored by fluorescence during amplification, and
  - (iv) NucSeqI' and NucSeqII' are localized on a single vector; and
- (3) determining from the results of the amplifications of step (2) the concentrations of NucSeqI and NucSeqII using the respective standard curves SC<sub>I</sub> and SC<sub>II</sub>, to obtain the **relative CN** of NucSeqI with respect to NucSeqII by the formula:

$$\text{Relative CN} = \frac{\text{Conc-I}_{\text{SCI}}}{\text{Conc-II}_{\text{SCII}}}$$

wherein, in said formula,

- (i) "relative CN" is the ratio of the CN of NucSeqI relative to the CN of NucSeqII in the sample;
- (ii) "Conc-I<sub>SCI</sub>" is the concentration of NucSeqI determined from standard curve SC<sub>I</sub>; and
- (iii) "Conc-II<sub>SCII</sub>" is the concentration of NucSeqII determined from standard curve SC<sub>II</sub>.

2. *(previously presented)* A method for determining the absolute CN of a nucleotide sequence NucSeqI in a sample, comprising:

- (a) determining the relative CN using the method of claim 18, and
- (b) multiplying the relative CN by the absolute CN of NucSeqII per cell.

3. *(previously presented)* A method according to claim 1, wherein at least two different NucSeqI' sequences used for measuring a corresponding number of different NucSeqI sequences are localized on a single vector.

4. *(previously presented)* A method according to claim 1 wherein the sequences of NucSeqI and NucSeqI' are the same.
5. *(previously presented)* A method according to claim 1 wherein the sequences of NucSeqII and NucSeqII' are the same.
6. *(previously presented)* A method according to claim 2, wherein at least two different NucSeqI' sequences used for measuring a corresponding number of different NucSeqI are localized on a single vector.
7. *(previously presented)* A method according to claim 2 wherein the sequences of NucSeqI and the NucSeqI' are the same.
8. *(previously presented)* A method according to claim 3 wherein the sequences of NucSeqI and the NucSeqI' are the same.
9. *(previously presented)* A method according to claim 6 wherein the sequences of NucSeqI and the NucSeqI' are the same.
10. *(previously presented)* A method according to claim 2 wherein the sequences of NucSeqII and the NucSeqII' are the same.
11. *(previously presented)* A method according to claim 3 wherein the sequences of NucSeqII and the NucSeqII' are the same.
12. *(previously presented)* A method according to claim 4 wherein the sequences of NucSeqII and the NucSeqII' are the same.
13. *(previously presented)* A method according to claim 6 wherein the sequences of NucSeqII and the NucSeqII' are the same.
14. *(previously presented)* A method according to claim 7 wherein the sequences of NucSeqII and the NucSeqII' are the same.
15. *(previously presented)* A method according to claim 8 wherein the sequences of NucSeqII and the NucSeqII' are the same.
16. *(previously presented)* A method according to claim 9 wherein the sequences of NucSeqII and the NucSeqII' are the same.

17. *(previously presented)* A method according to claim 1, wherein the sample is derived from cells.

18. *(previously presented)* A method according to claim 17, wherein an absolute CN of NucSeqII per cell is known.

19. *(previously presented)* A method according to claim 18, wherein at least two different NucSeqI' sequences used for measuring a corresponding number of different NucSeqI are localized on a single vector.

20. *(previously presented)* A method according to claim 18, wherein the sequences of NucSeqI and the NucSeqI' are the same.

21. *(previously presented)* A method according to claim 18 wherein the sequences of NucSeqII and the NucSeqII' are the same.

22. *(previously presented)* A method according to claim 19 wherein the sequences of NucSeqII and the NucSeqII' are the same.

23. *(previously presented)* A method according to claim 20 wherein the sequences of NucSeqII and the NucSeqII' are the same.

24. *(currently amended)* A method of determining the relative CN of a first nucleotide sequence I (NucSeqI) in a sample using an amplification technique, said method comprising the steps of:

- (1) adding to the sample nucleotides, primers, polymerase, a ~~fluorescently-labeled probe~~ probe directed to NucSeqI and NucSeqI' comprising a fluorophore and a quencher, and optionally, any additional reagents required for amplification, wherein the sample comprises a ~~chromosome-derived~~ second nucleotide sequence II (NucSeqII) and a probe directed to NucSeqII and NucSeqII' comprising a second fluorophore and a quencher, wherein the first fluorophore and the second fluorophore are different;
- (2) performing one or more amplification cycles to amplify NucSeqI, carrying out the following amplification steps:
  - (a) amplifying NucSeqI,
  - (b) amplifying NucSeqII,

- (c) amplifying a third nucleotide sequence I' (NucSeqI'), corresponding to NucSeqI and present in a control sample, at multiple dilutions, wherein the relationship of NucSeqI and NucSeqI' is defined as
  - (A) NucSeqI hybridizes to the complement of NucSeqI', and
  - (B) NucSeqI' hybridizes to the complement of NucSeqI,both under stringent hybridization conditions, and if NucSeqI and NucSeqI' differ in length, the shorter of the two is at most 30% shorter than the other;
- (d) amplifying a fourth nucleotide sequence II' (NucSeqII'), corresponding to NucSeqII and present in a control sample, at multiple dilutions, wherein the relationship of NucSeqII and NucSeqII' is defined as
  - (A) NucSeqII hybridizes to the complement of NucSeqII', and
  - (B) NucSeqII' hybridizes to the complement of NucSeqII,both under stringent hybridization conditions, and if NucSeqII and NucSeqII' differ in length, the shorter of the two is at most 30% shorter than the other;

wherein

- (i) the ratio of the concentration of NucSeqI' to the concentration of NucSeqII' is known,
  - (ii) standard curves SC<sub>I</sub> and SC<sub>II</sub> comprising at least two reference points are generated by amplification of NucSeqI' and NucSeqII', respectively, at multiple dilutions,
  - (iii) at least one pair of amplification reactions (a) and (b) or (c) and (d) is performed in a single container and monitored by fluorescence during amplification, and
  - (iv) NucSeqI' and NucSeqII' are localized on a single vector; and
- (3) determining from the results of the amplifications of step (2) the concentrations of NucSeqI and NucSeqII using the respective standard curves SC<sub>I</sub> and SC<sub>II</sub>, to obtain the **relative CN** of NucSeqI with respect to NucSeqII, by the formula:

$$\text{relative CN} = \frac{\text{Conc-I}_{\text{SCI}}}{\text{Conc-II}_{\text{SCII}}}$$

wherein, in said formula,

- (a) "relative CN" is the CN of NucSeqI relative to the CN of NucSeqII in the sample;

- (b) “Conc-I<sub>SCI</sub>” is the concentration of NucSeqI determined from standard curve SC<sub>I</sub>; and
- (c) “Conc-II<sub>SCII</sub>” is the concentration of NucSeqII determined from standard curve SC<sub>II</sub>.